with the application of the Tomsk regional common use center technical equipment acquired thanks to a grant of the Russian Ministry of the Agreement No. 14.594.21.0001 (RFMEFI59414X0001).

http://dx.doi.org/10.1016/j.ejcsup.2015.08.088

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Features of proteasome system functioning in the tumor and microenvironment cells of breast cancer

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Background: The tumor microenvironment plays an important role in the progression of cancer and may be regulated by proteolysis, including the proteasomes. The proteasomes modify the biologically important molecules involved in the pathogenesis and progression of a variety of malignancies, including breast cancer (BC). The aim of this study was to investigate the features of the subunit composition of proteasomes in the tumor and its microenvironment of breast cancer.

Material and Methods: The material for investigation was samples of tumor tissue of invasive ductal breast cancer. The study was conducted to estimate the distribution of the total pool of proteasome, proteasomes activator PA700, immune proteasome forms containing LMP7 and/or LMP2 subunit in tumor cells and stromal component using immunofluorescence. Furthermore, there was evaluated the distribution of the proteasome in the tumor. The expression of immune proteasomes and proteasomes activators in the cells was studied by immunofluorescence labeling of the cells by antibodies to immune proteasome subunits and cell markers. Fluorescence was analyzed by using a fluorescence microscope DM RXA2 ("Leica", Germany) and confocal microscope TCS SP ("Leica", Germany). The specificity of the primary antibodies was confirmed by check samples, at which the reaction was carried out only with the second antibody. No cross reaction between the first and second antibodies was tested by incubation of each primary antibodies with the opposing second antibodies. In addition, there was carried out labeling the cell nuclei by reagent Hoechst 33,342.

Results: It was found that the tumor cells comprise immune proteasomes, also PA700 and PA28αb activators. Activator PA28αb and immune proteasomes are localized in the cytoplasm of tumor cells, whereas α1, 2, 3, 5, 6, 7 subunits and PA700 activator detected in the cytoplasm and in the nuclei of tumor cells. Availability of α1, 2, 3, 5, 6, 7 subunits in the nuclei of tumor cells shows the expression of constitutive proteasome subunits. Stromal cells are characterized by a high ratio of the α1, 2, 3, 5, 6, 7 subunits to the immune LMP2 subunit as compared to cells of invasive ductal carcinoma. This means that the pool of proteasome in tumor cells of invasive ductal cancer is enriched by the immune proteasomes as compared to stromal cells. Thus, samples of invasive ductal breast cancer contain predominantly tumor cells enriched by immune proteasomes, activators PA700 and PA28αb. The presence of proteasomes in stromal component indicates that the tumor microenvironment also has active processes of proteolysis, with involving proteasome system. Probably the processes occurring in the stromal component contribute to the output of the proteasome into the extracellular space, which is confirmed by other researchers about the existence of circulating proteasome pools and their further participation in dissemination of cancer.

This work was supported by the Russian Foundation for Basic Research (Grant numbers 13-04-00169).

http://dx.doi.org/10.1016/j.ejcsup.2015.08.089

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Metformin in breast cancer therapy

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Background: Metformin is a antidiabetic drug with anticancer properties. However, the mechanism action by which metformin affects various cancer cells still unknown. It is known that tumor growth is accompanied by changes in the metabolic cascade that includes overproduction of lactate and adenosine. The adenosine is released into the extracellular environment and regulates differentiation, proliferation, and angiogenesis of tumor mass. We found that lactate is activator of key enzyme of adenosine metabolism – adenosine deaminase (ADA).

Aim: The aim of our study was to investigate the catabolism of adenosine in the tumor while taking metformin.

Materials and methods: In this study we investigated the level of adenosine, inosine, hypoxanthine and ADA activity in 15 women aged 46–76 years, with breast cancer (BC) T2-4N1M0 (cancer tissues) during treatment with metformin, 1000 mg per day for 3 months. Control group – 15 women aged 46–76 years, with stage T2-4N1M0 breast cancer (cancer tissues) without metformin therapy.

Statistical analysis was performed using the license package StatSoft. Statistica 12.0.

Results: ADA activity during treatment with metformin was 2-fold increased: 12.1 ± 2.49 nmol/min*mg in comparison with 4.77 ± 0.943 nmol/min*mg. Concentration of catabolic products of adenosine degradation was increased before metformin therapy. Inosine level was 0.121 ± 0.041 micro mol/g tissue (BC tissues from women without metformin 0.042 ± 0.015 micro mol/g tissue). Hypoxanthine 2.45 ± 0.428 micro mol/g tissue (in comparison with 0.711 ± 0.269 micro mol/g tissue). Whereas, adenosine level in BC after metformin therapy was 0.226 ± 0.148 micro mol/g tissue (in comparison with 0.186 ± 0.056 micro mol/g tissue), that were not significantly different.